# Gastric motor and sensory function in health assessed by magnetic resonance imaging: Establishment of reference intervals for the Nottingham test meal in healthy subjects

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Abstract: BACKGROUND Current investigations of gastric emptying rarely identify the cause of symptoms or provide a definitive diagnosis in patients with dyspepsia. This study assessed gastric function by magnetic resonance imaging (MRI) using the modular "Nottingham test meal" (NTM) in healthy volunteers (HVs). METHODS The NTM comprises (a) 400 mL liquid nutrient (0.75 kcal/mL) labeled with Gadolinium-DOTA and (b) an optional solid component (12 agar-beads [0 kcal]). Filling sensations were documented. MRI measurements of gastric volume, emptying, contraction wave frequency, and secretion were obtained using validated methods. KEY RESULTS Gastric function was measured in a population of 73 HVs stratified for age and sex. NTM induced moderate satiety and fullness. Labeled fluid was observed in the small bowel in all subjects after meal ingestion ("early-phase" GE). Secretion was rapid such that postprandial gastric content volume was often greater than meal volume (GCV0 > 400 mL), and there was increasing dilution of the meal during the study ($P < 0.001$). Gastric half-time was median 66-minutes (95% reference interval 35 to 161-minutes ["late-phase" GE]). The number of intact agar beads in the stomach was 7/12 (58%) at 60-minutes and 1/12 (8%) at 120-minutes. Age, bodyweight and sex had measurable effects on gastric function; however, these were small compared to inter-individual variation for most metrics. CONCLUSIONS AND INFERENCES Reference intervals are presented for MRI measurements of gastric function assessed for the mixed liquid/solid NTM. Studies in patients will determine which metrics are of clinical value and also whether the reference intervals presented here offer optimal diagnostic sensitivity and specificity.

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Gastric motor and sensory function in health assessed by magnetic resonance imaging: establishment of reference intervals for the Nottingham Test Meal in healthy subjects

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**Abstract**

Background: Current investigations of gastric emptying rarely identify the cause of symptoms or provide a definitive diagnosis in patients with dyspepsia. This study assessed gastric function by magnetic resonance imaging (MRI) using the modular "Nottingham Test Meal" (NTM) in healthy volunteers (HVs).

Methods: The NTM comprises (i) 400mL liquid nutrient (0.75kcal/mL) labelled with Gadolinium DOTA and (ii) an optional solid component (12 agar-beads (0kcal)). Filling sensations were documented. MRI measurements of gastric volume, emptying, contraction wave frequency and secretion were obtained using validated methods.

Key Results: Gastric function was measured in a population of 73 HVs stratified for age and sex. NTM induced moderate satiety and fullness. Labelled fluid was observed in the small bowel in all subjects after meal ingestion ("early-phase" GE). Secretion was rapid such that postprandial gastric content volume was often greater than meal volume (GCV0 >400ml) and there was increasing dilution of the meal during the study ($p<0.001$). Gastric half-time was median 66-Min (95% reference interval 35 to 161-Min ("late-phase" GE). The number of intact agar beads in the stomach was 7/12 (58%) at 60-Min and 1/12 (8%) at 120-Min. Age, body weight and sex had measurable effects on gastric function; however, these were small compared to inter-individual variation for most metrics.

Conclusions and Inferences: Reference intervals are presented for MRI measurements of gastric function assessed for the mixed liquid / solid NTM. Studies in patients will determine which metrics are of clinical value and also whether the reference intervals presented here offer optimal diagnostic sensitivity and specificity.

**Keywords:** Gastric emptying, gastric secretion, sensation, magnetic resonance imaging

**Key Messages**

- Current investigations of gastric function that focus on emptying rarely identify the cause of symptoms or provide a definitive diagnosis. This study provides comprehensive measurements of gastric function by magnetic resonance imaging (MRI) after ingestion of the mixed liquid/solid Nottingham Test Meal (NTM).
- Reference intervals for early- and late-phase liquid gastric emptying are established in a representative population of healthy volunteers. The presence of solids had little effect on gastric emptying of the liquid component.
- The NTM is well-tolerated and potentially suitable for assessment of gastric function by non-invasive imaging in clinical practice.

**List of Abbreviations:**

| | |
|---|---|
| GCV0 | Gastric content volume at time 0 min (mL) |
| GE | Gastric emptying |
| $GErateT_{50}$ | Gastric emptying rate at half emptying time (mL/min) |
| GSc | Gastric scintigraphy |
| GI | Gastrointestinal |
| HV | Healthy volunteer |
| Liquid-NTM | Liquid Nottingham test meal |
| Mixed-NTM | Mixed Nottingham test meal |
| NTM | Nottingham test meal |
| $T_{50}$ | Half emptying time |
| TGV | Total gastric content volume |

**Introduction**

Dyspeptic symptoms including uncomfortable fullness, early satiation, heartburn, nausea, bloating and epigastric pain account for up to 4% of primary care consultations and 25% of referrals to out-patient gastroenterology. [1-3] Clinical assessment and endoscopy fail to identify objective pathology in the majority of patients,[4] and in such patients the diagnosis of functional dyspepsia (FD) is made. The prevalence of FD in the community is estimated to be between 2.9% and 11.5 % depending on the diagnostic criteria applied. [5-9] Affected individuals have a reduced quality of life (QoL) [10-12] with impaired productivity at work and higher levels of absenteeism than healthy subjects [13, 14].

In patients with dyspeptic symptoms that do not respond to empiric management, gamma scintigraphy is recommended for assessment of gastric emptying (GE).[15, 16] Using the low fat "eggbeater" test meal delayed GE can be documented in approximately 40% of patients with FD and up to 75% of selected patients with severe, chronic unexplained nausea and vomiting. [17-19] However, the association of gastric emptying time with symptom severity is weak and delayed GE does not consistently predict clinical response to prokinetic and antiemetic medications.[20, 21] Thus, there is a need for investigations that can identify the causes of dyspeptic symptoms and direct effective therapy.[4, 22]

This study presents reference intervals for the assessment of gastric motor and sensory function by MRI after ingestion of the validated liquid Nottingham Test Meal (NTM).[23] This 400ml meal is approximately double the volume of the "egg beater" meal and contains sufficient calories and fat content (300kcal, 40% fat) to induce gastric fullness and satiety in healthy volunteers and dyspeptic sensations in FD patients.[23, 24] Further, analysis is not limited to a single measurement of gastric emptying (e.g. half time ($T_{50}$), residual volume at 2-4hours) [25-29]. Instead MRI measurements after a liquid meal can assess the increase in total gastric volume and the distribution of volume within the stomach (gastric relaxation or

"accommodation"), early and late phase gastric emptying, gastric secretion and motility.[29-31] At the same time, gastric sensitivity can be assessed by concurrent reporting of symptoms using validated scores.[32] A solid component comprised of agar beads with a known breaking strength can be added to the liquid-NTM. The break-down of this component provides an assessment of the mechanical work done by the antral contraction waves (trituration).[33] These measurements may be of relevance in FD, gastroparesis and other conditions characterized by the occurrence of symptoms during or immediately after meal ingestion [23, 24, 28-30, 34-37].

## Materials and Methods

### Participants

Adult healthy volunteers (HVs) aged 18 to 80 were recruited by advertisement. Subjects were stratified by age and sex so that a minimum of 10 men and women in three age groups (18-40, 41-60, 61-80) completed the Liquid-NTM. A subset of participants also completed the Mixed-NTM (Liquid and Solid components). Subjects underwent gastric scintigraphy and magnetic resonance imaging (MRI) on separate study days, a minimum of 48 hours apart. All study procedures were completed within a 4-month period. Reference intervals for gastric scintigraphy have already been reported in a previous publication.[23, 24]

At the initial screening visit, participants completed validated questionnaires regarding their health and wellbeing (patient health questionnaire (PHQ 12), hospital anxiety and depression questionnaire (HADS), EuroQol 5D™ (EQ-5D) [38-40]). Those invited to participate had no evidence of current medical problems, no functional gastrointestinal (GI) disease as defined by the Rome III Questionnaire or history of GI disease or surgery (other than appendicitis or hysterectomy) and no pathology on physical exam. Subjects were excluded if they had a waist circumference of over 100 cm and / or a body mass index (BMI) of less than 18 or over 30, took medication which may affect gastric motility for 7 days prior to investigation, had an

active eating disorder, vegan diet or allergy to milk protein. They had not participated in another radionuclide study within the previous 3 months or had any contraindication to MRI.

The protocol was approved by the NRES Committee East Midlands - Derby 1 and the Nottingham Research Ethics Committee 2. The study was registered at www.ClinicalTrial.gov (NCT01919021). Written informed consent was obtained from each participant. All procedures were performed in Nottingham University Hospital and the University of Nottingham, UK.

**Preparation**

Subjects fasted from midnight and abstained from alcohol and strenuous exercise for 24 h prior to each study day. Smoking was not permitted during the study.

**NTM preparation**

The Liquid-NTM comprised 400 mL vanilla Fortisip (Nutricia Clinical; Wiltshire, UK) diluted 1:1 with water (300 kcal, 11.6 g fat, 12 g protein, 36.8 g carbohydrates). A paramagnetic contrast agent (0.5 mmol/l Gadolinium-DOTA; Dotarem®, Guerbet, Aulnay-sous-Bois, France) was added to the Fortisip solution to increase contrast resolution and allow for gastric secretions to be calculated. Gadolinium-DOTA has been used extensively in studies of gastric function. The dose is an order of magnitude lower than that used in MRI angiography, it remains stable in acid environment and absorption is minimal from the gastrointestinal tract as determined by urine samples taken after test meals.[41] The Mixed-NTM contained the same liquid component. However, a solid component of the meal was added which comprised of 12 food grade agar beads prepared as originally described by Marciani et al. [42] with 1% Agar-Agar (Cuisine-innovation, Dijon, France) and 7.0 g/ 100 mL barium sulphate (E-Z-Paque: Buckinghamshire, UK Ph Eur 96% w/w). The barium was added to the agar beads to ensure that they remained negatively buoyant (i.e. did not float).

The breaking strength of the agar beads was 0.8 $N.m^{-2}$ as calculated by a tablet hardness tester (Erweka THB100, Heussentamm, Germany).

**Study Protocol**

Studies were performed with a 1.5T MRI system (Achieva, Philips, Best, The Netherlands). A 16-element phased-array, receive-only, flexible volume coil was placed around the abdomen in order to enhance signal detection (Sense XL Torso Coil 10, Philips, Best, The Netherlands). Gastric volume scans were determined from transverse balanced turbo field echo scans covering the stomach with 50 slices of 5 mm thickness, no slice gap, in plane resolution 2.0 x 1.7 mm2, Field of view 400 x 320 mm2, echo time/repetition time 1.5/3.0 ms, SENSitivity Encoding 2.0, FA 80°, data acquired in a short 16 s breath hold. Thin slices were used to reduce partial volume effects and a high flip angle was used to give good contrast between the fluid contents of the stomach and the surrounding walls. Motility scans to assess the frequency of antral contraction waves were performed at 15 and 75 min.[35] Gastric motility scans were carried out immediately after the dilution scans. Motility scans were acquired from 6-8 transverse oblique covering the luminal wall in the Liquid-NTM study. However, coronal scans were used in the Mixed-NTM study. Contraction Waves were recorded using a dynamic bFFE sequence accelerated with the parallel imaging technique sensitivity encoding (TR/TE = 2.7/1.37 ms; flip angle = 45°; SENSE reduction factor 2.0). A total of 40 dynamics were acquired for each subject over a period of 120 s using a navigator echo to reposition the slices during free breathing. Sequence parameters were as follows: slice thickness = 7 mm, matrix size = 160 x 160 (spatial resolution = 2.00 x 2.03 x 7.0 mm3). Dilution scans to assess secretion volume, based on validated methods for the Mixed-NTM were performed at 15 and 75 min after the volume scan [30, 37, 43].

*Liquid Nottingham Test Meal (Liquid-NTM)*

After baseline imaging, 200 mL of the liquid test meal was ingested from a series of beakers containing 50 mL liquid nutrient over 5 min. The subject was then imaged (-5 min scan). The remaining 200 mL of the test meal was then given in the same manner so that the entire test meal was consumed over 10 min and the subject imaged again (0 min scan). Gastric volume scans were then acquired at 5, 10, 15, 30, 45, 60, 75, 90, 120 min. After the 30 min time point, subjects were able to leave the scanner and remain in a seated waiting area. Images of the small bowel were then taken every 30 min until 240 min. At baseline and after each scan the subjects were asked to score satiety, fullness, bloating, heartburn, nausea and epigastric pain using a previously validated visual analogue scale (VAS 0–100 mm). [32]

*Mixed Nottingham Test Meal (Mixed-NTM)*

The first 200 mL of the liquid test meal was ingested as described above and the subject imaged (-5 min scan). The remaining nutrient drink was then given with 12 agar beads swallowed whole (3 beads with every 50 mL beaker). This two-stage methodology was required for the direct comparison with the GSc protocol used with the NTM, described previously in detail.[23, 24] Imaging continued for 120 min as for the liquid meal but with an additional 115 min time point. After the 115 min scan, 200 mL of water was given ahead of the final scan at 120 min so that the number of whole intact agar beads remaining within the stomach could be counted. No scans were acquired for the assessment of the small bowel. Gastric sensation was assessed as for the Liquid-NTM.

**Analysis**

*Gastric Sensation*

The following classification was applied for the analysis of all sensations documented by VAS: Mild <30, Mild-Moderate is 30-60, Moderate to Severe is 60-90, Severe is >90.

Fullness and Satiety were considered normal "filling" sensations. Bloating, Nausea, Abdominal Pain and Heartburn were considered pathological "dyspeptic" sensations.

*Gastric Volume*

Analysis of gastric volumes for the Liquid- and Mixed-NTM was performed as previously described.[23, 43] In brief, a semi-automatic method was used in order to outline the contents and air on each image slice. This required an intensity-based method to define both high signal intensity gastric content volume (GCV) and low signal intensity air volumes using a custom-written software (IDL version 6.4; Research Systems Inc., Boulder, CO, USA). The total gastric volume (TGV) was calculated from the sum of the air and content regions. The segmented area on each slice was multiplied by the slice thickness and summed over all contoured slices to measure the different stomach volumes (i.e. GCV, TGV including volume of agar beads (9.6mL) in mixed meal studies). The number of intact agar beads left in the stomach at 1 and 2 h was counted directly by the investigator from volume (axial) and coronal scans. Counting was aided by use of custom-written software (IDL 6.4), which allowed semi-automatic tracking of beads through the different slices.

*Antral Contraction and Secretion Volume*

Each dynamic antral contraction sequence was analysed frame by frame to follow the propagating indentations on the walls of the distal antrum. An axis was defined along the antral lumen in the direction of the propagation contraction wave. The frequencies of the antral contraction waves were then determined semi-automatically alongside the diameter (mm) of the antrum by established methods.[44] The frequency of antral contraction was presented as the number of contractions in 1-min. The Secretion volume analysis was performed as previously described by assessment of the dilution of the Gadolium-labelled meal for those subjects who underwent the Mixed-NTM study.[43]

**Statistical Analysis**

The number of patients required to assess reference interval for the liquid NTM study was calculated by a statistician at Trent Research & Development Support Unit based on the results of published pilot data and general criterion for sample size for reference intervals given by Harris & Boyd ("Statistical basis of reference values in laboratory medicine" Marcel Dekker. New York 1995). The calculation is based on the assumption that the 90% confidence interval for the reference limit is "small" compared with the 95% reference interval for the population. The width of the 95% reference interval is 2x1.96*s = 3.92s where s is the estimated standard deviation based on the results of published pilot data.[23] The target relative variation R = 5.62/(3.92√N), which, using a medium-sized value for R of 0.2 as a criterion for "small" yields a required sample size of 52 for reference interval of liquid gastric emptying by non-invasive imaging. Where insufficient data was present to calculate reference intervals, the confidence interval of the mean is presented (required to calculate statistical power in future studies). The number of subjects included in the mixed NTM meal had statistical power sufficient to detect a 20% difference in liquid emptying between the liquid- and mixed-NTM meals (alpha <0.05, beta >0.8).

Demographic results are reported as median with [Interquartile Ranges] and Wilcoxon tests were used for between group comparisons. Analysis of gastric motor and sensory function was performed by a specialist signal analyst and statistician (Menne Biomed Consulting, Tübingen, Germany). GCV and TGV data were fitted to LinExp curves with a Bayesian method using Stan source code as previously described. [24, 45] This method improved upon previously published model fits [23, 43] as the population-based approach assures that all curves can be fitted and give regularized coefficient estimates. The reference intervals were determined by the robust method as given in the Clinical and Laboratory Standards Institute's

and the ASVCP guidelines using a bootstrap method following outlier removal with the Horn algorithm, as implemented in function refLimit of the R package referenceIntervals (Finnegan (2014)) and reported as the upper and lower 95% reference intervals (RI) of the population [46]. The median was also provided alongside the upper and lower 95% confidence intervals (CI) of the mean. The reproducibility of MR volume data for the Liquid-NTM was determined by the repeatability coefficient ("rc") computed as \(1.96 * {SD}\), where SD is the standard deviation of the pairwise differences by the method of Bland and Altman. This is an approximate reference interval for within-subject repeats. For easier comparison, the normalized repeatability coefficient is also given. Within-subject difference of the GE parameters between the Liquid-NTM and Mixed-NTM was investigated with the Wilcoxon paired tests.

Bayesian model averaging was used to determine the effect of anthropometric factors and the addition of agar beads on the liquid GE parameters. This method accounts for model uncertainty inherent in the variable selection problem by averaging over the best models in the model class according to the approximate posterior model probabilities. [47, 48] A sensitivity analysis was performed to identify outliers for the covariate analysis. Identified outliers were manually removed from the analysis.

**Results**

*Participants*

A total of 91 subjects consented to the studies with 17 excluded due to excess weight (n=15) or concurrent use of medication (n=2) and one failed to attend a Mixed-NTM MRI study day. Therefore, 73 HVs completed the NTM studies (53 Liquid-NTM, 31 Mixed-NTM). 11 subjects completed both the Liquid-NTM study and the Mixed-NTM study. 9 subjects underwent the Liquid-NTM MRI study twice (repeat measurements were not included in the reference intervals).

Demographic, anthropometric and health questionnaire data for all subjects stratified by age and sex are provided in **Table 1**. A small number of subjects had a psychological disorder (n=4, HADS >11); however, self-rated health status was very good-excellent (>75 VAS in EQ-5D) in all subjects. There were no differences between the sub groups for either the HADS, PHQ or EQ-5D self-rated questionnaires **Table 1.** All subjects tolerated the complete 400 mL Liquid-NTM and Mixed-NTM.

*Measurement of gastric sensation*

There was no significant difference in the sensation of fullness between the liquid and mixed NTM meals ($p$=0.28); however, as presented in **Figure 1**, satiety tended to be higher after ingestion of the mixed meal ($p$=0.06). At baseline, most subjects reported minimal fullness (0 to 30 mm VAS). After completing the 400 mL test meal all subjects had some sense of fullness and satiety (i.e. >0 VAS) with 41/73 (56%) subjects reporting moderate fullness (i.e. >30 but <60 mm VAS). Fullness and other sensations often increased in the first 15-min of the postprandial period before decreasing steadily with GE. Mild - moderate bloating (>30 mm but <60 mm VAS) was reported by five (7%) of the healthy subjects. No other dyspeptic symptoms (i.e. nausea, heartburn, pain) were reported.

*Liquid gastric emptying*

*Reproducibility of gastric emptying*

For each parameter, reproducibility was assessed by the method of Bland and Altman in the 9 subjects who performed the Liquid-NTM MRI study twice (**supplementary table 1**). The lowest variance, corresponding to best repeatability, was present for initial volume (V0) and accommodation (normalized rc ~20%). The repeatability of half-emptying times was more variable (normalized rc ~40%).

*Reference Intervals of Liquid gastric emptying*

Gadolinium labelled meal was observed in the small bowel on the first scan in almost all patients. Notwithstanding this "early-phase" emptying, GCV0 measured immediately after NTM ingestion was often >400 mL due to secretion that occurred during meal ingestion (over 10 minutes). Subsequently there was a linear-exponential decrease in gastric meal volume over time (**Figure 2**). Liquid GE reference intervals for "early" and "late-phase" liquid emptying are presented in **Table 2a and 2b** for the Liquid- and Mixed-NTM respectively. Confidence intervals only are provided where insufficient data was present for calculation of reference intervals (see statistics section).

The number of whole intact beads (solid component) observed in the stomach after ingestion of the mixed NTM was median 7/12 (58%) at 60 and 1/12 (8%) at 120 min (**Figure 3**). The effects of the solid component on liquid emptying did not reach statistical significance (**Supplementary Table 2**). TGV0 tended to be larger (+45 mL (95% CI -5 to 82 mL, $p$=0.08)) with the Mixed-NTM than the Liquid-NTM due to the presence of the agar beads. The agar beads also tended to reduce liquid $T_{50}$ (-7 min (95% CI -18 to 3 min, $p$=0.08)). For most metrics confidence intervals for liquid emptying after ingestion of liquid (only) and mixed NTM overlapped to a large degree.

*Antral motility and diameter*

The mean frequency of antral contraction waves was 3/Min. There was no difference in the number of antral contractions between the Liquid and Mixed-NTM or between the 15 min and 75 min post meal ingestion time point ($p$=0.57 and $p$=0.79 respectively), **Supplementary Table 3.** The mean antral diameter at the 15 min time point for the Liquid-NTM was 31 mm (95% CI 29 to 33 mm). This was 5 mm smaller than the antral diameter measured with the Mixed-NTM ($p$=0.02).

*Gastric Secretion*

Gastric dilution scans to assess gastric secretion were performed in the 31 subjects who underwent the Mixed-NTM and sufficient data was available to analyze results for 28 subjects at 15 and 29 patients at 75 minutes respectively. Missing secretion volume data was due to scanner operator error. At 15 minutes, median GCV was 404 (374 to 428) mL with median secretion volume 70 (55 to 88) mL (approximating to 17% (14 to 22%) GCV). At 75 min median GCV was 192 (161 to 248) mL with median secretion volume 97 (70 to 122) mL (approximating to 50% (33 to 64%) GCV). Thus, secretion volume was higher at 75 min than 15 min ($p$=0.011) and there was increasing dilution of the meal during the emptying process ($p<0.001$). Additionally, the distribution of secretion was not homogeneous throughout the stomach with a much higher level of dilution within a secretion layer above the meal in the proximal stomach than observed in the distal stomach ($p<0.001$).

*Effect of patient factors on gastric function*

Bayesian model averaging (BMA) was used to determine the effect of demographic and anthropometric factors on the GE parameters GCV0, $T_{50}$ and GErate$T_{50}$ for both GCV and TGV, **Supplementary Table 3**. There was no single predictor of early phase gastric emptying (GCV0); however, increasing age was associated with a small increase in TGV0 (14.3 mL for every 10 y of age, posterior probability 95%). After removal of three outliers with a $T_{50}$ of over 150 min, there were no single predictors for either GCV $T_{50}$ or TGV $T_{50}$. For late phase emptying, GCV GErate$T_{50}$ was associated with body weight such that, with every 10kg weight increase GCV GErate$T_{50}$ increases by 0.2 mL/min (posterior probability of 67%). Additionally, the TGV GErate$T_{50}$ was faster by 0.65 mL/min with male sex (posterior probability of 68%).

## Discussion

This study provides reference intervals for the clinical assessment of gastric motor and sensory function by magnetic resonance imaging (MRI) for the modular "Nottingham Test Meal" (NTM) from a representative cohort of healthy participants (**Table 1**). Measurements of total gastric volume (TGV) and gastric content volume (GCV), antral contraction wave frequency and gastric secretion are presented after ingestion of the 400ml liquid NTM with and without the solid NTM component (non-nutrient agar beads). Reports of fullness and satiety after meal ingestion confirmed that the size of the meal was sufficient to induce normal postprandial sensations in the majority of healthy controls (**Figure 1**).

### *Liquid Gastric Filling and Emptying*

A characteristic pattern of volume change was observed for TGV and GCV after ingestion of the liquid nutrient meal (**Figure 2**). As in previous reports,[23, 29] the gastric content volume (GCV fasted ($GCV_f$)) and total gastric volume ($TGV_f$) at baseline before ingestion of the meal were small (median 20 mL and 53 mL, respectively). Similarly, with the exception of three elderly patients, the increase in the volume of air in the stomach during meal ingestion was relatively small (~100ml) and subsequently remained remarkably constant throughout the study. As a result, changes in GCV after meal ingestion (gastric filling ($GCV_{Acc}$)) were closely associated to changes in TGV (gastric relaxation or "accommodation" [*] ($TGV_{Acc}$)) during NTM ingestion and subsequent emptying.

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[*] Accommodation is defined differently when assessed by different measurement techniques. Conceptually it refers to tonic relaxation of the stomach during a meal which allows gastric filling without an excessive increase in gastric pressure. For imaging studies the increase in gastric volume during a meal is used as a surrogate of this process. Fasting and postprandial gastric volumes were measured by this study. Reference intervals for gastric accommodation ($V_{Acc}$) are provided in table 2.

Gastric emptying (GE) commenced already during ingestion of the liquid NTM as evidenced by the presence of Gadolinium labelled liquid in the small bowel in the first image acquired after meal ingestion. This "early-phase" emptying was followed by a "late-phase" linear–exponential reduction in meal volume (**Figure 2**). Analysis of volume data shows that (i) it is not appropriate to normalize volume after meal ingestion because a substantial *and variable* amount of emptying (5-25% of meal volume [23, 24]) has already occurred at this time point and (ii) it is not adequate to describe GE using a single measurement (e.g. $T_{50}$, retention time at 2 hours). These observations are consistent with previous Magnetic Resonance Imaging (MRI) studies that show "early phase" GE is related only to the volume load ingested (gastric relaxation or "accommodation"), whereas "late phase" GE is modulated by volume and calorie load (tonic contraction modulated by neurohormonal feedback).[29, 30, 35-37] Physiological studies have shown rapid early phase GE (low GCV0) after ingestion of a large liquid meal in functional dyspepsia (FD) patients with impaired gastric accommodation documented by gastric barostat;[31, 35] however, in many FD patients, this is followed by relatively slow late phase GE (low GE$rateT_{50}$).[28] This biphasic pattern may be caused by rapid, "early phase" delivery of liquid nutrient into the small bowel leading to disproportionate activation of the "neurohormonal brake" with subsequent slow, "late phase" emptying. Similar "fast then slow" emptying patterns have been observed in food engineering studies that manipulate particle size to modulate GE, nutrient absorption and satiety.[49-51] Thus, ingestion of food that has been finely blended or that forms fine protein precipitates or fat emulsions in the stomach is followed by rapid early-phase GE and absorption of nutrients that triggers neurohormonal feedback that slows subsequent, late-phase GE and prolongs gastric retention times.[49-51] Ongoing clinical studies will assess the prevalence of this pattern of emptying in FD patients.[28] If present, then this finding may identify a subgroup of patients that respond to specific dietary or pharmacological interventions that slow early-phase GE

and nutrient absorption and/or the release of peptide hormones (e.g. cholecystokinin (CCK)) that modulate gastric motor and sensory responses, including the generation of dyspeptic symptoms after meal ingestion.[52-54]

*Gastric Secretion*

MRI measurements of gastric content volume (GCV) include not only the meal but also gastric secretions. The volume of gastric secretions independent of the meal can be estimated by measuring the dilution of the paramagnetic contrast agent in the liquid meal.[37, 43] Using this approach it was observed that the rate of gastric secretion during meal ingestion can be greater than the rate of emptying at the beginning of the study, such that GCV0 was > 400ml (i.e. greater than NTM volume) in approximately half the subjects. Gastric secretion continued during the study, such that meal dilution increased from 20% GCV at 15 minutes to 50% GCV at 75 minutes after meal digestion. Additionally, as described in previous studies,[55, 5657] the distribution of secretion was not homogeneous, with a much higher level of dilution in the proximal stomach than in the distal stomach. The capability of this technology to provide non-invasive measurements of gastric secretion could be of value in clinical medicine. Both the absolute volume of gastric secretions and the collection of unbuffered secretion in the proximal stomach (often termed the "acid pocket" [57]) are pathological factors in conditions such as peptic ulcer and gastro-oesophageal reflux disease.

*Gastric Motility*

The frequency of antral contraction waves was approximately 3/min and did not change during the course of the study. Antral motility can be quantified by calculating a motility index which integrates the frequency and vigor of contraction.[44, 58, 59] It is known that patients with gastroparesis have a lower motility index compared with healthy volunteers.[60, 61] In contrast, the motility index of patients with pyloric obstruction (pylorospasm or peptic

pyloric stenosis) is higher than that of the volunteer group.[61] Moreover, Ajaj and colleagues demonstrated that the gastric motility index in patients with gastroparesis increased, whereas in the group with functional pyloric obstruction it decreased after appropriate therapy.[61]

*Gastric Trituration and the Effect of Solids on Gastric Function*

The Solid Component of the NTM comprised 12 agar beads with known breaking strength. Marciani et al. have shown that the rate by which these beads break down provides a direct measurement of work done by antral contraction waves (i.e. trituration).[42] In the mixed NTM, non-nutrient material was selected to minimize effects of the solid component on liquid GE and other measurements.[23] The findings confirm that the presence of the agar beads had only small effects on gastric motor or sensory function and confidence intervals for liquid emptying after ingestion of liquid (only) and mixed NTM overlapped to a large degree. This confirms that the liquid NTM can be used with or without the modular solid component. The number of whole, intact beads in the stomach reduced in a linear fashion from 12 to median 7 (58%) at 60-min and 1 (8%) at 120-min (**Figure 3**). The breakdown of solids into small particles (generally <3mm) is a pre-requisite for gastric emptying after meals;[62, 63] however, it must be noted that the rate of trituration does not necessarily equate to the rate of solid emptying from the stomach.[23]

*Gastric sensation*

After completing the 400 mL NTM all subjects reported an increase in fullness and satiety and 41/73 (56%) subjects reported moderate fullness (i.e. >30 but <60 mm VAS). In many subjects the sensation of fullness continued to increase in the early postprandial period, before steadily falling as GE occurred (**Figure 1**). This finding confirms that the volume and composition of the NTM is sufficient to trigger normal postprandial sensations in HVs. Mild

- moderate bloating was reported by five (7%) of the healthy subjects. No other dyspeptic symptoms (i.e. nausea, heartburn, pain) were reported.

During the study the sense of fullness correlated linearly with changes in gastric volumes. This observation is consistent with the findings of previous MR studies,[42, 64] and supports the hypothesis that it is not meal volume alone, but total gastric volume (i.e. meal, secretion and air) that determines sensation in HVs. This has been shown to be the case also in a randomized controlled study that compared effects of aerated drinks (foams) of differing gastric stability on gastric volume and appetite, compared with a control drink. [65]

*Effect of age, sex and weight on gastric function*

As summarized in **supplementary table 4**, there was no single demographic or anthropometric predictor of early phase gastric emptying (GCV0) or GE half time ($T_{50}$); however, increasing age was associated with a progressive increase in TGV0 suggesting increased gastric compliance / accommodation in older subjects. For late-phase GE, the rate of emptying (GCV GE$rateT_{50}$) increased with body weight (0.2 mL/min with every 10kg weight increase). This finding is consistent with a recent report from Blümel and colleagues that subjects with low body weight empty more slowly than those with normal or increased body weight.[66]

*Limitations*

This study provides reference intervals for liquid gastric emptying from a large, representative population of healthy individuals (n=73). Confidence intervals are provided for other metrics where insufficient data to calculate reference intervals was present. The Clinical Laboratory Standard Institute has recommended a minimum of 120 patients to establish normal values in a system with large inter-individual variation and a large degree of physiological redundancy.[46] An alternative approach is to apply this data alongside patient

data to determine thresholds that define not the "normal range", but conclusively pathological function. It should be noted that some of the measurements presented in this study may be redundant; however, this will become clear only when clinical studies are performed. Further limitations include the relative high level of variability for certain measurements (**supplemental table 1**) and the complexity of gastric volume change after meals, in particular the impact of secretion on measurements of gastric filling and emptying. This study obtained independent measurements of meal and secretion volumes by measuring the dilution of the Gadolinium marker in the meal;[43] however, this requires additional scans and may not be feasible in routine practice. Finally, the NTM is not typical of a normal meal. Most meals are heterogeneous with liquid and solid components that empty at different rates and issues such as mastication rates or layering of fats within the stomach can have important effects on gastric emptying.[67-69] The use of homogenous liquid and solid components for the NTM limits the impact of many of these, potential, confounding factors and allows independent assessment of multiple gastric functions; however, as in all clinical investigations, although simplification makes the test easier to perform and analyse it also makes it less physiological.

*Conclusion and Potential application in clinical practice*

Reference limits for gastric function have been obtained using standard MRI procedures without the need for specialized equipment. At present image analysis is time consuming; however, semi-automated methods are in development that would greatly facilitate measurement of gastric volume data.[70] Otherwise, there are few barriers to implementation of this technology in clinical practice. Indeed, the ability to assess multiple aspects of gastric function by MRI provide an attractive alternative to existing, invasive diagnostic tools (e.g. antro-pyloro-duodenal manometry) for diagnosis of gastric and gastrointestinal motility disorders and therapeutic monitoring.[71]

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## Tables

**Table 1** Demographic, anthropometric and health questionnaire data for healthy volunteers by age and sex reported as the median and [interquartile range]. Wilcoxon tests were used for between group comparisons of sex stratified groups. .

| | n | Age | Height | Weight | BMI | Waist C | PHQ | HADS | EQ5D |
|---|---|---|---|---|---|---|---|---|---|
| **18 to 40 f** | 16 | 21.0 [20.0, 26.0] | 1.6 [1.6, 1.7] | 59.9 [57.8, 68.5] | 23.2 [21.4, 25.4] | 79.5 [70.8, 86.2] | 2.0 [1.0, 2.5] | 3.5 [1.8, 5.5] | 90.0 [82.2, 95.0] |
| **41 to 60 f** | 11 | 47.0 [45.0, 52.0] | 1.6 [1.6, 1.7] | 66.0 [63.5, 68.8] | 24.2 [23.6, 26.0] | 86.0 [82.0, 88.5] | 2.0 [1.5, 3.0] | 4.0 [2.2, 5.0] | 95.0 [90.0, 100.0] |
| **Over 60 f** | 13 | 67.0 [65.0, 75.0] | 1.6 [1.6, 1.6] | 60.7 [55.9, 71.9] | 25.4 [21.0, 27.5] | 87.0 [74.0, 95.0] | 2.0 [1.0, 6.0] | 5.0 [3.0, 7.0] | 98.0 [90.0, 100.0] |
| **18 to 40 m** | 12 | 20.5 [20.0, 21.0] | 1.8 [1.8, 1.8] | 71.0 [66.8, 74.3] | 21.8 [20.4, 24.0] | 81.0 [78.0, 82.0] | 1.0 [0.0, 1.1] | 2.5 [1.0, 4.0] | 88.8 [78.8, 93.1] |
| **41 to 60 m** | 11 | 47.0 [46.0, 51.5] | 1.8 [1.8, 1.8] | 78.0 [74.7, 91.0] | 25.4 [24.1, 28.1] | 93.0 [89.0, 98.0] | 1.5 [0.5, 2.5] | 4.5 [2.5, 7.0] | 90.0 [82.5, 93.5] |
| **Over 60 m** | 10 | 67.0 [64.8, 68.0] | 1.7 [1.7, 1.8] | 76.8 [73.2, 87.0] | 25.6 [24.3, 27.6] | 92.5 [89.2, 97.5] | 2.2 [2.0, 3.8] | 7.5 [2.8, 8.9] | 90.0 [86.2, 90.0] |
| ***p* value** | | | | <0.001 increase with age | 0.009 increase with age | <0.001 increase with age | 0.079 | 0.247 | 0.031 |

BMI, body mass index (kg/m$^2$); EQ5D, Euroqol 5D™; f, female; HADS, hospital anxiety and depression score; m, male; n, number; PHQ, patient health questionnaire, *p* value derived Wilcoxon test between group comparison of sex stratified groups and Waist C, waist circumference (cm).

**Table 2.** Reference intervals of the liquid gastric emptying parameters for (A) Liquid- and (B) Mixed-Nottingham Test Meal (NTM).

**Table 2A**

| Gastric emptying parameter | Median | 95% Confidence interval of mean | 95% Reference interval of population | Outliers (n) removed from calculation |
|---|---|---|---|---|
| GCVf (mL) | 20 | 18…24 | 3-49 | 3 |
| TGVf (mL) | 52 | 44…58 | 8-127 | 2 |
| $GCV_0$ (mL) | 424 | 415…433 | 388…500 | 2 |
| $TGV_0$ (mL) | 524 | 510…545 | 419…675 | 2 |
| $GCV_{Acc}$ (mL) | 404 | 395…412 | 342…464 | 2 |
| $TGV_{Acc}$ (mL) | 472 | 460…492 | 370…556 | 2 |
| GCV $T_{50}$ (min) | 66 | 59…71 | 37…144 | 2 |
| $TGVT_{50}$ (min) | 68 | 59…72 | 41…132 | 6 |
| GCV $GErateT_{50}$ (mL/min) | 2.5 | 2.4…2.9 | 1.4…4.6 | 2 |
| TGV $GErateT_{50}$ (mL/min) | 3.1 | 3.0…3.5 | 1.8…5.1 | 3 |

**Table 2B**

| Gastric emptying parameter | Median | 95% Confidence interval of the mean | 95% Reference interval of population | Outliers (n) removed from calculation |
|---|---|---|---|---|
| GCVf (mL) | 20 | 18…24 | 3-49 | 3 |
| TGVf (mL) | 52 | 44…58 | 8-127 | 2 |
| $GCV_0$ (mL) | 451 | 436…461 | 398…530 | 2 |
| $TGV_0$ (mL) | 553 | 530…574 | 458…708 | 1 |
| $GCV_{Acc}$ (mL) | 420 | 408…436 | 354…480 | 2 |
| $TGV_{Acc}$ (mL) | 496 | 472…516 | 412…588 | 1 |
| GCV $T_{50}$ (min) | 66 | 61…72 | 43…118 | 1 |
| $TGVT_{50}$ (min) | 59 | 56…66 | 39…107 | 1 |
| GCV $GErateT_{50}$ (mL/min) | 2.7 | 2.6…3.0 | 1.9…4.1 | 1 |
| TGV $GErateT_{50}$ (mL/min) | 3.5 | 3.2…3.7 | 2.3…5.2 | 0 |

GCV, gastric content volume (meal volume and secretion); TGV, total gastric content volume (meal volume, secretion volume and air volume). Vf fasting volume (ml); V0, volume at time 0 min (mL) after meal ingestion - low values indicate rapid early phase emptying; VAcc increase in gastric volume after meal compared to baseline (V0-Vf); $T_{50}$, half emptying time; GErateT50, gastric emptying rate at T50 (mL/min) – low values indicate slow late phase emptying. Note: Since fasting volume is independent of meal volume, baseline data from all records where pooled and is the same in both groups.

**Figure Legends**

**Figure 1** Reported visual analogue scores over time after ingestion of Liquid-NTM or Mixed-NTM. Fullness and Satiety increased from the start of the study (-10 Min) with ingestion of 200ml (-5 Min) and 400ml (0 Min). From that point there was a steady return of scores towards baseline. The mean and 95% confidence intervals of the mean are provided. Panel A: Fullness and Panel B: Satiety. Light grey bar indicates Liquid-NTM and dark grey bar indicated Mixed-NTM. NTM; Nottingham test meal.

**Figure 2** Liquid gastric emptying reference intervals of the Liquid-NTM and Mixed-NTM. The outer ribbon represents the 95% reference interval of the population and the inner ribbon represents the bootstrapped 95% confidence interval of the mean. Panel A; Liquid-NTM and Panel B; Mixed-NTM. NTM: Nottingham test meal.

**Figure 3** The number of whole intact agar beads in the stomach at 60 and 120 min for the Mixed-NTM. Solid black line indicates the median and boxes indicate the quartile range. NTM: Nottingham test meal.

**Supporting information**

**Supplementary Table 1**

Reproducibility of measurements assessed by the method of Bland and Altman based on within-subject standard deviation and means of 9 subjects. SD is the standard deviation of the pairwise differences, rc is the 95% repeatability coefficient, and norm_rc is the normalized repeatability.

| **Parameter** | **Mean** | **SD** | **rc** | **norm_rc** |
|---|---|---|---|---|
| GCVf (mL) | 17 mL | 10 | 18 | 108% |
| TGVf (mL) | 45 mL | 35 | 68 | 149% |
| GCV0 | 421 mL | 45 | 87 | 21% |
| TGV0 | 523 mL | 46 | 92 | 18% |
| GCVAcc | 400 mL | 40 | 80 | 20% |
| TGVAcc | 480 mL | 40 | 78 | 16% |
| GCV T50 | 72 min | 16 | 32 | 43% |
| TGV T50 | 73 min | 15 | 29 | 40% |
| GCV GErateT50 | 2.6 mL/min | 0.9 | 1.7 | 65% |
| TGV GErateT50 | 3.1 mL/min | 1.0 | 2.0 | 66% |

GCV: gastric content volume, TGV: total gastric volume; Vf: volume at baseline (fasted); V0: volume at time 0 min (mL), VAcc: change in volume after meal ingestion from baseline (Accommodation); $GErateT_{50}$; gastric emptying rate at $T_{50}$ (mL/min), NTM; Nottingham test meal, $T_{50}$; half emptying time (min),

**Supplementary Table 2**

Within-subject difference between Liquid-NTM and Mixed-NTM tested with Wilcoxon paired tests (positive difference indicates larger measurement for Mixed-NTM meal). Ingestion of agar beads tended to increase gastric relaxation (accommodation) and tended to increase the rate of GE (lower $T_{50}$, faster $T_{50}$ $GERateT_{50}$); however, the effect size was relatively small and confidence intervals overlapped for all metrics.

| **Parameter** | **Difference** | **P Value** | **95% Confidence interval of the mean** |
|---|---|---|---|
| GCV0 | +14 mL | 0.17 | -10…+41 |
| TGV0 | +47 mL | 0.08 | -5…+82 |
| $GCV_{Acc}$ | +16 mL | 0.05 | +12…+24 |
| $TGV_{Acc}$ | +24 mL | 0.04 | +12…+48 |
| GCV T50 | -5 min | 0.28 | -16…+6 |
| TGV T50 | -7 min | 0.07 | -15…+2 |
| GCV GErateT50 | +0.5mL/min | 0.15 | -0.4…0.9 |
| TGV GErateT50 | +0.6mL/min | 0.10 | -0.1…1.0 |

GCV; gastric content volume, GCV0; gastric content volume at time 0 min (mL), $GERateT_{50}$; gastric emptying rate at $T_{50}$ (mL/min), NTM; Nottingham test meal, $T_{50}$; half emptying time (min) of liquid Fortisip, TGV; total gastric content volume and TGV0; total gastric content volume at time 0 min (mL).

**Supplementary Table 3**

Antral contraction and antral diameter reference intervals of the Liquid and Mixed Nottingham test meal.

| NTM Meal | Measure | Time point | n | Median | 95% Confidence interval of the mean | 95% Reference interval of the population |
|---|---|---|---|---|---|---|
| Liquid | Antral Contraction | 15 | 50 | 3.0 | 2.9…3.2 | 2.1…4.0 |
| Liquid | Antral Contraction | 75 | 15 | 3.1 | 3.0…3.4 | NA* |
| Mixed | Antral Contraction | 15 | 20 | 3.2 | 2.9…3.4 | 2.0…4.3 |
| Mixed | Antral Contraction | 75 | 19 | 3.2 | 2.8…3.3 | NA* |
| Liquid | Antral Diameter | 15 | 50 | 33 | 29…33 | 14…48 |
| Mixed | Antral Diameter | 15 | 20 | 36 | 32…39 | NA* |

N; the number of subjects, NTM; Nottingham test meal and NA* reference intervals were not calculated due to smaller number of subjects.

**Supplementary Table 4**

Bayesian model averaging of the effect of anthropometric factors on the Nottingham test meal gastric emptying parameters. The predictive models were tested and the results from the best three alternate models are provided for each parameter. The posterior probability is relative to all of the models compared. * indicates that the analysis was performed without 3 outliers.

| **Parameter** | Models (n) | | **p!=0 (%)** | **EV** | **SD** | **Model 1** | **Model 2** | **Model 3** |
|---|---|---|---|---|---|---|---|---|
| GCV0 (mL) | 7 | Age | 5 | 0.004 | 0.043 | . | . | . |
| | | Waist | 10.1 | 0.047 | 0.184 | . | . | 0.467 |
| | | Height | 9.5 | 4.119 | 16.98 | . | . | . |
| | | Weight | 18.8 | 0.096 | 0.239 | . | 0.509 | . |
| | | Male sex | 5.1 | 0.171 | 1.772 | . | . | . |
| | | BMI | 7.7 | 0.097 | 0.482 | . | . | . |
| | | **Posterior probability** | | | | 0.439 | 0.188 | 0.101 |
| TGV0 (mL) | 7 | Age | 95.1 | 1.158 | 0.465 | 1.43 | 1.13 | 1.2 |
| | | Waist | 17.5 | 0.262 | 0.696 | . | . | . |
| | | Height | 31.5 | 51.08 | 89.22 | 173.2 | . | . |
| | | Weight | 26.9 | 0.358 | 0.706 | . | 1.36 | . |
| | | Male sex | 21.9 | 5.700 | 13.41 | . | . | 29.7 |
| | | BMI | 5.2 | -0.074 | 1.066 | . | . | . |
| | | **Posterior probability** | | | | 0.17 | 0.147 | 0.11 |
| *$T_{50}$ (min) GCV | 12 | Age | 16.2 | 0.0272 | 0.0753 | . | . | . |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Waist | 7 | 0.014 | 0.1094 | . | . | . |
| | | Height | 13.8 | -4.895 | 14.615 | . | -35.579 | . |
| | | Weight | 21.5 | -0.073 | 0.1728 | . | . | -0.291 |
| | | Male sex | 9.5 | -0.565 | 2.1738 | . | . | . |
| | | BMI | 4.3 | -0.022 | 0.1876 | . | . | . |
| | | **Posterior probability** | | | | 0.331 | 0.138 | 0.125 |
| *$T_{50}$ (min) TGV | 12 | Age | 5.7 | 0.0040 | 0.0287 | . | . | . |
| | | Waist | 4.4 | 0.0003 | 0.0426 | . | . | . |
| | | Height | 13.1 | -3.896 | 12.400 | . | . | -29.67 |
| | | Weight | 8.5 | -0.016 | 0.0716 | . | . | . |
| | | Male sex | 15.1 | -0.902 | 2.6026 | . | -5.97 | . |
| | | BMI | 4.6 | -0.009 | 0.1523 | . | . | . |
| | | **Posterior probability** | | | | 0.417 | 0.151 | 0.131 |
| GErate$T_{50}$ GCV (mL/min) | 14 | Age | 7.6 | -0.001 | 0.0016 | . | . | -0.00414 |
| | | Waist | 7.2 | -0.001 | 0.0044 | . | . | . |
| | | Height | 22.8 | 0.450 | 0.9815 | . | 2.34486 | . |
| | | Weight | 79.2 | 0.0183 | 0.0119 | 0.02277 | . | 0.02328 |
| | | Male sex | 10.2 | 0.0264 | 0.1049 | . | . | . |
| | | BMI | 12.6 | 0.0019 | 0.0193 | . | . | . |
| | | **Posterior probability** | | | | 0.418 | 0.098 | 0.076 |
| | | | **p!=0 (%)** | **EV** | **SD** | **Model 1** | **Model 2** | **Model 3** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| GErateT$_{50}$ (mL/min) TGV | 14 | Age | 3.4 | 0.001 | 0.0009 | . | . | . |
| | | Waist | 3.8 | 0.001 | 0.0023 | . | . | . |
| | | Height | 19.8 | 0.001 | 1.1604 | . | . | . |
| | | Weight | 31.7 | 0.001 | 0.0121 | . | 0.0266 | 0.0153 |
| | | Male sex | 68.2 | 0.001 | 0.3294 | 0.6525 | . | 0.4459 |
| | | BMI | 16.6 | 0.001 | 0.0238 | . | . | . |
| | | **Posterior probability** | | | | 0.284 | 0.125 | 0.124 |

EV; Bayesian model average posterior mean, GCV; gastric content volume; GCV0; gastric content volume at time 0 min (mL), gastric emptying rate GErateT$_{50}$ ;gastric emptying rate at T$_{50}$ (mL/min), n; number of subjects, NTM; Nottingham test meal, Models (n); the number of models used to predict a relationship, P!=0; the probability that the variable contributes to the model, , SD; Bayesian model average posterior standard deviation T$_{50}$, half empting time (min) of liquid Fortisip, TGV; total gastric content volume and TGV0; total gastric content volume at time 0 min (mL).